



Applicants Paul B. Fisher and Malavi T. Madireddi
U.S. Serial No.: 09/515,369
Filed : February 29, 2000
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A3
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CONT.

AP-1 or C/EPB wild type or mutant oligonucleotides (AP-1/WT; 5'-CGCTTGATGACTCAGCCGGAA-3'), (SEQ ID NO: 5), (C/EBP/WT; 5'-TGCAGATTGCGCAATCTGC A-3'), (SEQ ID NO. 6), (AP-1/MT; 5'-CGCTTGATGACTTGGCCGGAA-3') (SEQ ID NO:7) and C/EBP (C/EBP/MT; 5'-TGCAGAGACTAGTCTCTGCA-3') (SEQ ID NO:8) or 1-5 µg of either anti-cJun/AP-1 or anti-C/EPB-β antibody (SantaCruz) were added to the binding reactions along with the labeled probe and reactions were incubated for 30 min at RT prior to electrophoresis. The gels were then dried and exposed to X-ray film.

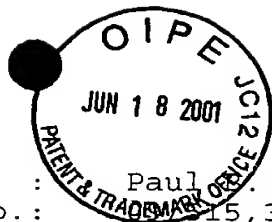
REMARKS

The specification has been amended to include reference to SEQ ID NOs. An annotated version of the amended paragraphs of the specification showing all changes relative to the previous version of that paragraph is attached hereto as **Exhibit D**.

The May 29, 2001 Notice states that the subject application does not comply with the Sequence Rules and that applicants must provide 1) a substitute computer readable form (C.R.F.) copy of the "Sequence Listing"; 2) an substitute paper copy of the "Sequence Listing", and 3) a statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. §1.821(e) or §1.821(f) or §1.821(g) or §1.825(b) or §1.825(d).

In response to the May 29, 2001 Notice, applicants submit herewith a computer readable form (CRF) of the Sequence Listing.

Applicants further submit a paper copy of the Sequence Listing, attached hereto as **Exhibit B**, and a Statement in Accordance with 37



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C.F.R. §1.821(f), attached hereto as **Exhibit C**, certifying that the computer readable form as required by 37 C.F.R. §1.821(e) is identical to the paper copy of Sequence Listing attached as **Exhibit B**.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorneys invite the Examiner to telephone at the number provided below.

No fee is deemed necessary in connection with the filing of this Amendment. However, if any other fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

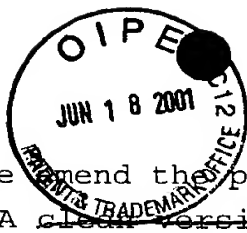
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I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:

Assistant Commissioner for Patents
Washington, D.C. 20231.

John P. White
Reg. No. 28,678

Date



Please amend the paragraph on page 3, line 23 through page 4, line 11. A ~~clean~~ version of the amended paragraph follows:

Marked up

(Amended) **Figures 2A-2C.** Determination of *mda-7* transcription by nuclear run-on, *mda-7* mRNA by Northern blotting and RT-PCR and a comparison of the AU-rich sequences found in the 3'-UTR of several mRNAs. (Figure 2A) Nuclear run-on assays using nuclei isolated from Control or 24 h after treatment with IFN- β , MEZ or IFN- β + MEZ, the same concentrations as indicated in Fig. 1. GAPDH was used as an internal control. In vitro transcription assays were performed as previously described (Jiang et al., 1993). (Figure 2B) *Mda-7* message expression detected by Northern blotting and RT-PCR followed by Southern blotting. Total RNA from control (Figure 2C) HO-1 cells and cells treated with IFN- β (I), MEZ (M) or IFN- β +MEZ (I+M) were analyzed by Northern blotting or RT-PCR/Southern using radiolabeled *mda-7* cDNA as a probe as previously described (Jiang et al., 1993, 1995a; Kang et al., 1998a). GAPDH was used as an internal loading control. (C) Several cytokine genes and proto-oncogenes that contain the AUUUA consensus sequence in their 3'-UTRs. Abbreviations: Hu = human; *mda-7* = melanoma differentiation associated gene-7 (Jiang et al., 1995a); α -IFN = alpha interferon (Goeddel et al., 1981); GM-CSF = granulocyte-monocyte colony stimulating factor (Wong et al., 1985); TNF = tumor necrosis factor (Nedwin et al., 1985); cFos = fos proto-oncogene (van Straaten et al., 1983). (SEQ ID NOS:[1-5]9-13).

Please amend the paragraph on page 57, line 25 through page 58, line 5. A ~~clean~~ version of the amended paragraph follows:

Marked up

(Amended) ***Isolation and cloning of the mda-7 promoter.*** A human placental genomic l library (Stratagene) was screened using the *mda-7* cDNA (18) labeled by random priming (Life Technologies, Inc.) using α -³²P[dCTP]. Three l phage clones were identified and isolated



to homogeneity. An anti-sense primer, 5'-CGTCCCAGCCGTGGAAGTCAT-3' (SEQ ID NO:2) corresponding to the region 40-50 bp from the 5' terminal end of the *mda-7* cDNA was used with the T3 or T7 primer in a polymerase chain reaction to amplify the region upstream of the *mda-7* cDNA from the three 1 phage clones. The proof reading polymerase, Tth polymerase (Clonetech) was employed for this purpose. One of the 1 phage clones yielded a 2.2 Kbp amplification product that was cloned into pBluescript and sequenced (ABI sequencing).

Please amend the paragraph on page 59, line 20 through page 60, line 12. A ~~clean version~~ of the amended paragraph follows:

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(Amended) **Electrophoretic Mobility Shift Assay (EMSA)**. Nuclear extracts were prepared as described (25). Briefly, binding reactions were performed in 10 or 20 ml reaction mixtures containing 1-3 mg of nuclear extracts from control or differentiation inducer treated HO-1 cells. The binding buffer contained 12 mM HEPES (pH 7.9), 5 mM MgCl₂, 60 mM KCl, 0.6 mM EDTA, 0.5 mM dithiothreitol, 1 mg of poly (dI-dC), 10% glycerol. The region corresponding to the putative AP-1 and C/EBP binding sites present between NdeI and NheI restriction enzyme sites was PCR amplified using flanking primers, 5'-AGGCTGGATTG GCTTGTGAC-3' (Sense) (SEQ ID NO:3) and 5'-CTGTTTAATCCAGCACTTCCC-3' (Antisense) (SEQ ID NO:4). The PCR product was column purified (Qiagen), end labeled with γ -³²P [ATP] and 1500 cpm of double stranded DNA were used per binding reaction. Binding reactions were performed at RT for 30 min. Reactions were then loaded onto a 4% polyacrylamide gel and electrophoresed at 4° C at 100 V in 0.25X Tris-borate-EDTA as described (26,27). Competition and supershift reactions were identical to those described above, except a 10-100 fold excess of AP-1 or C/EBP wild type or mutant oligonucleotides (AP-1/WT; 5'-CGCTTGATGACTCAGCCGGAA-3'), (SEQ ID NO: 5), (C/EBP/WT; 5'-TGCAGATTGCGCAATCTGC A-3'), (SEQ ID NO. 6), (AP-1/MT; 5'-CGCTTGATGACTTGGCCGGAA-3') (SEQ ID NO:7) and C/EBP (C/EBP/MT; 5'-



TGCAGAGACTAGTCTGCA²) (SEO ID NO:8) or 1-5 μ g of either anti-cJun/AP-1 or anti-C/EPB- β antibody (SantaCruz) were added to the binding reactions along with the labeled probe and reactions were incubated for 30 min at RT prior to electrophoresis. The gels were then dried and exposed to X-ray film.